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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/776,635	02/12/2004	John J. Rossi	1954-418	1769

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EXAMINER	
BOWMAN, AMY HUDSON	

ART UNIT	PAPER NUMBER
1635	

DATE MAILED: 04/26/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/776,635	ROSSI ET AL.	
	Examiner	Art Unit	
	Amy H. Bowman	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 February 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4 and 6-26* is/are pending in the application.

4a) Of the above claim(s) *9,10 and 26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 4, 6-8, and 11-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of Application/Amendment/Claims

Applicant's response filed 2/1/2006 has been considered. Rejections and/or objections not reiterated from the previous office action mailed 9/1/2005 are hereby withdrawn. The following rejections and/or objections are either newly applied or are reiterated and are the only rejections and/or objections presently applied to the instant application.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 2, 3, and 5 have been cancelled. Claims 1, 4, and 6-26 are pending in the application. Claims 9, 10 and 26 have been withdrawn as being drawn to a nonelected invention.

This application contains claims 9, 10 and 26 drawn to an invention nonelected with traverse on 07/21/05. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Claim Objections

Claim 11 is objected to because of the following informalities: Claim 11 recites "a antisense". Amendment of the claim to read "an antisense" would obviate this objection. Appropriate correction is required.

Response to Arguments--Claim Rejections - 35 USC § 112

Claims 1, 4, 6, 7, 8, 11-17 and 19-25 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for exposing a cell to an siRNA molecule and screening for methylation *in vitro*, does not reasonably provide enablement for siRNA directed methylation *in vivo*, for the reasons of record set forth in the office action mailed 9/1/2005.

Applicant has cancelled claim 2, obviating the rejection against this claim.

Applicant argues that the Caplen reference was published more than three years prior to the present application and that the state of the art has advanced significantly since then. Applicant argues that the very authors of Caplen et al. have shown subsequently to the reference relied upon by the examiner that sequence-specific dsRNA mediated interference of gene expression has been observed in mammalian cells. The examiner has not asserted that sequence-specific dsRNA mediated interference of gene expression has not been observed in mammalian cells, but rather that the *in vivo* delivery of such compounds is unpredictable and faces several delivery challenges that render such delivery unpredictable. Simply because Caplen et al. may have achieved delivery in mammalian cells does not render such delivery to be predictable.

Applicant cites art to support *in vivo* delivery of siRNA compounds. Applicant points to Castanotto et al. for teachings of RNAi in mammalian cells using siRNA or shRNA. It is unclear what the relevance of this teaching is to the instant argument because the examiner has not argued delivering siRNA to mammalian cells, but rather

has argued the predictability of delivering siRNA to mammalian cells ***in vivo***. Castanotto et al. teach transfection of mammalian cells in 6-well plates *in vitro*. As explained in the office action mailed 9/1/2005, the instant application is enabled for *in vitro* delivery of siRNA compounds to mammalian cells.

Applicant relies upon the teachings of Zhou et al. to demonstrate that siRNA is effective for sequence specific suppression of heterologous and endogenous gene expression in Xenopus embryos. Zhou et al. teach microinjection of a Xenopus expression vector, which is not representative of the instant scope which is drawn to exposing any mammalian cell to a siRNA molecule by any means of delivery. Furthermore, the examiner does not dispute the fact that siRNA duplexes have been delivered *in vivo*, but rather that such delivery is unpredictable due to many delivery challenges, for the reasons explained in the office action mailed 9/1/2005.

Applicant points to the teachings of Calegari et al. for similar results in mouse embryos. Calegari et al. teach injection of siRNAs into the lumen of the neural tube and delivery into neuroepithelial cells by electroporation. This topical injection followed by electroporation in mammalian postimplantation embryos is not representative of the instant scope which is drawn to exposing any mammalian cell to a siRNA molecule by any means of delivery.

Applicant points to the teachings of McCaffrey et al. and Lewis et al. for similar results in mice. McCaffrey teaches injection of siRNAs to mice. This art relied upon by applicant teaches that although the results show that siRNAs are functional in mice, delivery remains a major obstacle (see column 1 of page 39). Lewis et al. teach rapid

injection of siRNAs into the tail veins of mice. High-pressure tail vein injection is not representative of the instant scope which is drawn to exposing any mammalian cell to a siRNA molecule by any means of delivery. Furthermore, McCaffrey acknowledges that delivery remains a major obstacle for siRNAs.

Applicant points to Paddison et al. for teaching that short hairpin RNA is effective for inducing gene silencing in mammalian somatic cells. Paddison et al. teach that shRNAs were somewhat less potent than siRNAs and that roughly 50% of each were competent for suppressing gene expression. Paddison et al. further teach that neither analysis of the predicted structures of the target mRNA nor analysis of alternative structures in siRNA duplexes or shRNA has proved of predictive value for choosing effective inhibitors of gene expression (see column 2 of page 951). These teachings of Paddison et al. are considered to support the unpredictability of siRNA delivery. Applicant points to a passage of Paddison et al. that teaches that shRNAs can be provided exogenously or can be synthesized *in vivo* from RNA polymerase promoters (see page 956). However, the actual shRNAs or siRNAs taught by Paddison et al. were produced *in vitro* and the cells taught by Paddison et al. were transfected with siRNAs or shRNAs in 6-well plates (see Materials and methods, page 956, column 2 and page 957, column 1), which is not representative of the instant scope which encompasses exposing any mammalian cell to an siRNA molecule *in vivo* by any means of delivery.

Applicant argues that Matthieu et al. is directed to a study of RNA-directed DNA methylation in plants and explains differences between the process in plants and animals. The delivery of siRNA duplexes to both mammalian and plant cells was known

in the art at the time the invention was made. The topic of contention is whether such delivery is predictable *in vivo*. Whether the art teaches such delivery in mammalian or plant cells, the mechanism of DNA methylation is not well understood and the delivery of siRNA duplexes is unpredictable. The differences that applicant explains between the processes occurring in mammalian and plant cells are considered irrelevant to the unpredictability of delivery of siRNA duplexes because siRNA duplexes have not proven to predictably overcome delivery challenges in either system.

Mathieu et al. (Journal of Cell Science, 2004, 117(21), pp. 4884-4888) teach that the mechanism by which RNA signals are translated into DNA methylation imprints is currently unknown. Applicant argues that they are not required to determine and set forth the precise mechanism of DNA methylation. It is noted that the examiner did not require the applicant to do so. The examiner cited this teaching from Mathieu et al. as support for the lack of knowledge regarding the DNA methylation pathway. There is a lack of knowledge in the art regarding DNA methylation *in vivo*. This deficiency is not compensated for in the instant specification. Therefore, delivery of siRNA molecules *in vivo* to achieve the instantly desired effect is neither routine nor predictable. Furthermore, *in vivo* delivery of siRNA duplexes for purposes of inhibiting the expression of a target gene faces many challenges, as supported by the art relied upon by the examiner. Delivery of these same duplexes to methylate a gene of interest would face the same exact challenges and unpredictability.

Applicant argues that there are techniques known in the art to determine a siRNA to utilize, to determine the form of the oligonucleotide, to determine the mode of

delivery, the amount of oligonucleotide to be delivered, and the length of time of the viability of the oligonucleotide. Applicant argues that although these techniques may require some experimentation for a given target sequence, such experimentation is not undue. On the contrary, each one of these variables would have to be empirically determined for each oligonucleotide duplex. Due to the unpredictability of *in vivo* delivery of siRNA duplexes, one could not practice the invention commensurate in scope with the claims without undue, trial and error experimentation.

Response to Arguments-- Claim Rejections - 35 USC § 103

Claims 1, 4, 6-8, 22 and 25 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Elbashir et al. (Nature, 2001, Vol. 411, pp. 494-498), in view of Mette et al. (The EMBO Journal, 2000, Vol. 19, No. 19, pp. 5194-5201), further in view of Dammann et al. (Nature Genetics, 2000, Vol. 25, pp. 315-319), for the reasons of record set forth in the office action mailed 9/1/2005.

Applicant has cancelled claim 2, obviating the rejection against this claim.

Applicant argues that the art relied upon by the examiner does not teach all elements of the invention. Applicant argues that there is no suggestion in Elbashir et al. of directing siRNA molecules to target regions that are within a promoter region. Applicant argues that this deficiency of Elbashir et al. is not taught by Dammann et al. The examiner did not rely upon Elbashir et al. or Dammann et al. for such a teaching, but rather relies on Mette et al. for teaching targeting dsRNA duplexes to a promoter. Therefore, the combination of the references as cited teach each element of the

instantly recited invention. Applicant argues that the promoter methylation taught by Mette et al. is not a PTGS process but is a TGS process and that each of these are distinct pathways in plants. As taught by Mette et al., both transcriptional and post-transcriptional gene silencing can be initiated by double-stranded RNAs that enter the same degradation pathway (see abstract).

Applicant argues that Mette et al. is directed to plants which differ from animals. It is noted that the Mette et al. reference is relied upon for teaching the concept that the promoter is a good target for dsRNA mediated DNA methylation. Therefore, whether the teachings of Mette et al. are regarding plants or animals is irrelevant to this argument. Mette et al. teaches targeting dsRNA in plants, whereas Elbashir et al. teaches targeting siRNA duplexes in mammal cells. Each of these were known in the art at the time the invention was made. Since targeting a promoter in the RNAi pathway was known at the time the invention was made, motivation and reasonable expectation of success are present. Applicant's arguments regarding PTGS may or may not be true, but are not considered germane to the argument at hand. Mette et al. certainly teaches a correlation by teaching RNAi as a PTGS process in which dsRNA induces the degradation of homologous RNA sequences (see introduction). Mette et al. clearly teaches that the dsRNA is implicated as directing DNA methylation.

It would have been obvious to one of ordinary skill in the art to expose a cell to a siRNA which is specific for a target sequence in order to methylate a gene of interest in a mammalian cell because of the coupled teachings of Mette et al. and Elbashir et al.

New Rejections

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 11-21, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Elbashir et al. (Nature, 2001, Vol. 411, pp. 494-498), in view of Mette et al. (The EMBO Journal, 2000, Vol. 19, No. 19, pp. 5194-5201), for the reasons of record set forth in the office action mailed 9/1/2005 and maintained above, further in view of Tuschl (Nature Biotechnology, May 2002, Vol. 20, pp. 446-448), Castanotto et al. (RNA, 2002, 8, pp. 1454-1460), and Peel et al. (Journal of Neuroscience Methods, 2000, 98, pages 95-104).

The invention of the above claims is drawn to a method for methylating a gene of interest in a mammalian cell comprising exposing said cell to an siRNA molecule which is specific for a target sequence in said gene of interest, wherein said target sequence is located in a promoter region of said gene of interest and wherein said siRNA directs methylation of said gene of interest. The cell is exposed to said siRNA by introducing into said cell DNA sequences encoding a sense strand and an antisense strand of said siRNA, wherein said siRNA is expressed in the cell. The introducing is accomplished by using at least one vector, wherein the vector is a plasmid vector, an adeno-associated vector, or a viral vector, more specifically a retroviral vector. The introducing takes

place *in vivo* or *in vitro* and is achieved via transformation, transfection, infection, or via a liposome. The DNA sequences are generated by PCR and are in the same or separate vectors.

Elbashir et al. and Mette et al. do not teach exposure of a siRNA by introducing into a cell DNA sequences encoding a sense strand and an antisense strand of a siRNA. Elbashir et al. and Mette et al. do not teach vectors or liposomes.

Tuschl teaches that several transient methods of siRNA transfer into cells have been utilized in the art including liposome-mediated transfection, electroporation, and microinjection. Tuschl teaches that specific genes have been targeted by intracellular expression of siRNAs from plasmid DNA. Tuschl teaches that intracellular transcription of small RNA molecules can be achieved by cloning the siRNA templates into RNA polymerase III transcription units, which normally encode the small nuclear RNA U6 or the human RNAse P RNA H1. Tuschl teaches that sense and antisense strands constituting the siRNA duplex can be transcribed by individual promoters or siRNAs can be expressed as fold-back stem-loop structures that give rise to siRNAs after intracellular processing. Tuschl teaches that the endogenous expression of siRNAs from DNA templates is thought to overcome some limitations of exogenous siRNA delivery. Tuschl teaches that incorporation of siRNA expression cassettes into retroviral vectors may also allow the targeting of primary cells previously refractory to siRNA or plasmid DNA transfection. Tuschl discusses experiments that were carried out *in vitro* that mediated RNAi.

Castanotto et al. teach functional siRNA expression from transfected PCR products. Castanotto et al. teach a PCR strategy to yield U6 transcription cassettes expressing siRNAs.

Peel et al. teach that adeno-associated virus vector may provide sufficient spatio-temporal control of gene expression. Peel et al. teach that advantages to utilizing an adeno-associated virus vector are efficiency, longevity, and lack of toxicity.

It would have been obvious to one of ordinary skill in the art to expose the siRNA of Elbashir et al. to a cell by introducing into DNA sequences encoding a sense strand and an antisense strand of the siRNA, as taught by Tuschl. It would have been obvious to utilize a plasmid vector, retroviral vector, or liposome, as well as to utilize transfection, as taught by Tuschl to deliver and produce the siRNA taught by Elbashir et al. Further, it would have been obvious to utilize an adeno-associated vector as taught by Peel et al. to deliver the siRNA of Elbashir et al. Additionally, it would have been obvious to generate the DNA sequences by PCR, as taught by Castanotto et al.

One would have been motivated to expose the siRNA of Elbashir et al. to a cell by introducing DNA sequences encoding a sense and an antisense strand of the siRNA because Tuschl teaches that specific genes have been targeted by intracellular expression of siRNAs from plasmid DNA and further teaches that the endogenous expression of siRNAs from DNA templates is thought to overcome some limitations of exogenous siRNA delivery.

One would have been motivated to utilize liposome-mediated transfection to deliver the siRNA of Elbashir et al. because Tuschl teaches that this method is

commonly utilized in the field to deliver siRNAs to a cell. One would have been motivated to utilize a plasmid, retroviral, or adeno-associated vector to deliver the siRNA of Elbashir et al. because Tuschl teaches using plasmids and teaches that incorporation of siRNA expression cassettes into retroviral vectors may also allow the targeting of primary cells previously refractory to siRNA or plasmid DNA transfection, and Peel et al. teach that adeno-associated virus vectors yield advantages such as increasing efficiency, longevity, and lack of toxicity.

Finally, one would have been motivated to generate the DNA sequences via PCR because Castanotto et al. teach functional siRNA expression from transfected PCR products. Castanotto et al. teach a PCR strategy to yield U6 transcription cassettes expressing functional siRNAs.

One would have a reasonable expectation of success given that each of the elements were known in the art at the time the invention was made to add benefits to forming and delivering siRNA duplexes, as evidenced by Tuschl and Castanotto et al. One would reasonably expect for an adeno-associated virus as taught by Peel et al. to benefit the siRNA taught by Elbashir et al. because adeno-associated viruses were known to benefit the delivery of oligonucleotides and to offer benefits such as increased efficiency, longevity, and lack of toxicity.

Thus in the absence of evidence to the contrary, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amy H. Bowman whose telephone number is 571-272-0755.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance.

Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public. For more information about the PAIR system, see http://pair-direct.uspto.gov.

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